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Systemic mast cell activation disease: the role of molecular genetic alterations in pathogenesis, heritability and diagnostics

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Summary

Despite increasing understanding of its pathophysiology, the aetiology of systemic mast cell activation disease (MCAD) remains largely unknown. Research has shown that somatic mutations in kinases are necessary for the establishment of a clonal mast cell population, in particular mutations in the tyrosine kinase Kit and in enzymes and receptors with crucial involvement in the regulation of mast cell activity. However, other, as yet undetermined, abnormalities are necessary for the manifestation of clinical disease. The present article reviews molecular genetic research into the identification of disease-associated genes and their mutational alterations. The authors also present novel data on familial systemic MCAD and review the associated literature. Finally, the importance of understanding the molecular basis of inherited mutations in terms of diagnostics and therapy is emphasized.

Keywords: heritability; mast cell activation disease; mast cell activation syndrome; mastocytosis; molecular genetics.

Introduction

The term systemic mast cell activation disease (MCAD; synonym mast cell disease) refers to disorders characterized by an enhanced release of mast cell mediators sometimes accompanied by accumulation of such dysfunctional mast cells which may or may not be readily histologically detectable (ref. 1 and further references therein). According to the current classification[1–3] three variants of systemic MCAD are distinguished: systemic mastocytosis (SM), mast cell activation syndrome (MCAS) and mast cell leukaemia (MCL). The first of these, SM, is characterized by specific pathological mutations and immunohistochemical findings, which are known as the World Health Organization criteria.[4] A diagnosis of MCAS[1–3,5,6] is assigned to patients who present with multiple mast cell mediator-induced symptoms but who do not fulfil the requirements for a diagnosis of SM, and in whom relevant differential diagnoses have been excluded. The last, MCL, is an aggressive mast cell neoplasm which is defined by increased numbers of mast cells in bone marrow smears ($\geq$ 20%) and the presence of circulating mast cells (reviewed in ref. 4).

Few data are available concerning the prevalence of systemic MCAD. Both SM and MCL are rare disorders.[4,7] For SM, data from the French mastocytosis network Association Française pour les Initiatives de Recherche sur le Mastocyte et Les Mastocytoses,[8] the Spanish mastocytosis network Red Española de Mastocitosis,[9,10] the Italian Mastocytosis Registry,[11] and the German Competence Network on Mastocytosis (own unpublished results), suggest a prevalence of at least one in 364 000 in Europe. However, given that these data represent only a proportion of all cases, the true prevalence will be higher. Data from a clinical population suggest that the prevalence of MCL is two orders of magnitude lower than that of SM.[12] In contrast, MCAS seems to be a more common disorder. Evidence has been presented that MCAS may be an underlying cause of various clinical presentations, e.g. in subsets of patients with fibromyalgia[13] and irritable bowel syndrome.[14] Hence, the prevalence of MCAS is likely to lie within the single-digit percentage range.

The relationship between systemic MCAD and cutaneous mastocytosis (CM, synonyms: paediatric or childhood-onset mastocytosis) remains unclear. Early studies suggested that CM and systemic MCAD were separate dis-

Abbreviations: CM cutaneous mastocytosis; GNNK glycine-asparagine-asparagine-lysine; IL-4 interleukin 4; MCAD mast cell activation disease; MCAS mast cell activation syndrome; MCL mast cell leukaemia; SM systemic mastocytosis

ease entities, because the majority of CM patients were found to lack mutations of the tyrosine kinase *KIT* gene.[15,16] However, subsequent studies have demonstrated that the frequency of clonal *KIT* mutations is similar in patients with CM, SM and MCAS, and that they are present in up to 86% of patients from each diagnostic group.[17–20] In addition, several studies have reported the evolution of CM into SM, suggesting that the two disorders are not distinct, but may instead be part of a continuous spectrum of mast cell-related dysfunction.[18,21–24] However, unless otherwise stated, the present review is confined to discussion of the systemic MCAD variants in the definitions of the current consensus, i.e. SM, MCAS and MCL.

Genetic alterations in patients with systemic MCAD

The establishment of an aberrant clonal mast cell population is dependent upon mutations in genes that encode proteins with a crucial involvement in the regulation of mast cell activity. Those studied best are the mutations occurring in the tyrosine kinase Kit.[18,25–27] In the majority of patients with systemic MCAD, these mutations are limited to derivatives of haematopoietic stem cells.[28,29] This indicates that these mutations did not arise during early embryonic development, and were therefore somatic rather than germline. The mutations underlying systemic MCAD drive aberrant mediator production/release with or without readily histologically detectable mast cell accumulation. Mast cell accumulation is due predominantly to a decrease in mast cell apoptosis (refs 30,31 and further references therein). On a limited scale, it is also due to an increase in proliferation.[32,33] The mutations underlying MCL appear to drive malignant mast cell proliferation in addition to aberrant mediator production and release.[4,34,35]

A general view on genetic alterations in tyrosine kinase Kit

The type-III transmembrane receptor tyrosine kinase Kit plays a crucial role in the development of mast cells, as well as of haematopoietic progenitor cells, melanocytes, primordial germ cells and the interstitial cells of Cajal (for review, see ref. 36). In humans, the *KIT* gene (previously termed *c-kit*), which encodes the tyrosine kinase Kit, is located in the pericentromeric region of the long arm of chromosome 4 (4q12; http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene&cmd=retrieve&dopt=default&list_uids=3815#ubor2_RefSeq). The human *KIT* gene spans approximately 89 kb and contains 21 exons, which are transcribed/translated into a protein with a molecular mass of 145 000 and a length of 976 amino acids (http://atlasgeneticsoncology.org/Genes/KITID127.html). Under physiological conditions, binding of the endogenous Kit ligand, i.e. the stem-cell factor, to the extracellular domain of the receptor results in receptor dimerization, activation of the intracellular tyrosine kinase domain through autophosphorylation of specific tyrosine residues, and receptor activation (for review, see ref. 37). In normal mature mast cells, activation of Kit signalling through stem-cell factor leads to increased cell proliferation and survival, changes in mast cell migration and adhesion, mast cell degranulation and mediator release.

Various somatic heterozygous Kit alterations (point mutations and deletions/insertions) have been detected in patients with systemic MCAD (Table 1). In any given patient, one Kit alteration or a combination of two or more Kit alterations can occur. For several of these genetic alterations of *KIT* (Table 1, bold typed) it has already been demonstrated that they cause changes in the downstream Kit signalling pathways in mast cells. These convert Kit into a constitutively active, dysregulated, stem-cell-factor-independent tyrosine kinase. Independence of stem-cell factor binding may be explained, at least in part, by kinase activity within unusual mutation-induced subcellular locations, such as the Golgi compartment or the endoplasmic reticulum.[38,39] Abnormalities in the growth, proliferation and survival of mutated mast cells are mediated by activation of the phosphoinositide 3-kinase/protein kinase B/mammalian Target of Rapamycin pathway. Research suggests that enhancement of mast cell degranulation is mediated by tyrosine phosphorylation of non-T-cell activation linker.[40] Within this context, it is interesting to note that within any given patient, mutations that are functionally activating in mast cells may be functionally inactivating in other Kit-expressing cells, e.g. in the Cajal cells of the gastrointestinal tract.[41] This may be a result of differences in the signalling cascades. This could explain why a minority of patients with systemic MCAD present with constipation rather than diarrhoea.[1]

The described Kit isoforms

Six Kit isoforms exist as a result of the presence of three alternative splicing sites. Each isoform is of differing pathophysiological relevance. An additional glutamine residue at amino acid position 252 is present because of the insertion of an alternative splicing consensus sequence at the 3′ end (− 5 nucleotides) of intron 4, at the beginning of the coding sequence of exon 5.[25] The two isoforms, i.e. 252Q(+) and 252Q(−), are present in similar numbers in patients with MCAD and healthy volunteers (Figure 1),[25,26] which suggests that they have no crucial involvement in the induction of an unregulated increase in mast cell activity.

Two further isoforms result from alternative 5' splice donor sites,[42] and these are characterized by the presence or absence of the tetrapeptide sequence glycine-asparagine-asparagine-lysine (GNNK) in the extracellular part

Table 1. Distribution of alterations in the amino acids sequence of the tyrosine kinase Kit (deduced from the nucleotide sequence of the 21 exons of the gene *KIT*) in mast cells from patients with systemic mast cell activation disease (mast cell activation syndrome and systemic mastocytosis).

					Signal transduction exons 12–21, aa 592–976		
Ligand(SCF)-binding domain exons 1–5 aa 1–308	Dimerization domain exons 6–7 aa 309–410	Proteolytic cleavage site exons 8–9 aa 411–513	Membrane-spanning region exon 10 aa 514–549	Auto-inhibition exon 11 aa 550–591	Kinase domain1 Kinase insert sequence,	Kinase domain 2	C-terminus
W8R[1]	D327N[2]	D419H/G[2]	***Del 510–513***[1,2,5]	E554K[2]	Del 592–626[1]	F782S[1]	Frequently splicing errors[1,2]
C12S[1]	E338K[1,2]	**419InsFF**[3]	Del 521[1]	**V559I/A/G**[6,10]	G658E[2]	N787D[1]	
L18P[2]	Q346L[1]	**Del 419**[3]	**F522C**[6]	**V560G**[2,6,11]	Y672S[2]	H790R[1]	
P31T[2]	M351E/I[1,2]	**Del 417–419InsY**[3]	V530I[7]	**D572A**[3]	683 Ins R[2]	H802Y[2]	
41 Stop codon[1]	F355L[1]	**C443Y**[3]	**A533D**[6,8]		S688L[2]	814 Stop codon[2]	
E53K[1,2]	E359V[1]	S464L[2]	**M541L**[1,2,3,8,9]		S709A[1]	A814V/T[13]	
Del59–437[1]	359 Stop codon[1]	475 Stop codon[1,2]			*Del 715*[1,2,12]	R815K[6]	
E73R[1]		**S476I**[3]			E720K[2]	Ins V815-I816[6]	
T74R[1]		K484R[2]			M724I[2]	**D816V/Y/F/H/I**[3,6,11]	
K116N[2]		**ITD501–502**[3]			A736V[1]	I817V[6]	
V214L[2]		**ITD502–503**[3]			D751Y[1]	D820G[14]	
252Ins Q[1,2]		**ITD505–508**[3]			D760V[2]	S821F[1]	
K259E[1]		**K509I**[3,4]			E761K[2]	**N822I/K**[15,16]	
H265Q[1]					764 Stop codon[2]	A829T[1]	
E270K[1,2]						830 Stop codon[2]	
L276S[1]						A837V[1]	
G286R[2]						E839K[6]	
						L862V[1,2]	

[1]Ref. 26; [2]Ref. 25; [3]Ref. 18; [4]Ref. 68; [5]Ref. 43; [6]Ref. 27; [7]Ref. 74; [8]Ref. 17; [9]Ref. 19; [10]Ref. 64; [11]Ref. 75; [12]Ref. 45; [13]Ref. 76; [14]Ref. 77; [15]Ref. 20; [16]Ref. 78.

Three isoforms resulting from alternative splicing are indicated by italic type. Mutations which have been demonstrated to induce an increased mast cell activity are indicated by bold type. aa, amino acid; SCF, stem cell factor.

of the juxtamembrane region. *In vitro*, these isoforms differ substantially in terms of their functional activities, with the GNNK(−) isoform showing tumorigenic potency.[43,44] Hence, a predominance of the GNNK(−) isoform may contribute to or reflect a pathological increase in the activation of affected mast cells. Studies comparing MCAD patients with healthy controls have reported significantly stronger expression of the GNNK (−) isoform compared with the GNNK(+) isoform in mast cells from patients with MCAD (Figure 1).[26] An expression intensity ratio for GNNK(−)/(+) of $\geq$ 80% was associated almost exclusively with high symptom intensity. Although the reason for the predominant expression of the GNNK(−) isoform in these patients is unknown, its presence appears to support a diagnosis of MCAD. However, the absence of this predominant expression does not exclude the diagnosis.

The third alternative splicing site in KIT results in the presence or absence of a serine residue at position 715 in the kinase insert region of human Kit. This results from an alternative 3' splice acceptor site usage.[45] The two isoforms S715(−) and S715(+) are co-expressed in similar quantities in patients with MCAD and in healthy controls (Figure 1).[25,26] This suggests that these isoforms have no substantial involvement in the induction of a pathological increase in mast cell activity.

The mutation Kit^{D816V}

In the mast cells of the majority (> 90%) of patients with SM, mutations in the activation loop of Kit are present (most frequently D816V; reviewed in ref. 27). According to recent studies, the pathological immunohistochemical alterations that constitute the World Health Organization criteria (formation of mast cell clusters; spindle-shaped morphology of mast cells; expression of CD25 in mast cells; marked increase in blood tryptase concentration) seem to be both causally related and specific to the occurrence of a mutation at amino acid position 816 of tyrosine kinase Kit in the affected mast cells.[4,46,47] During SM progression, the Kit mutant D816V may disappear (ref. 48 and own unpublished observation).

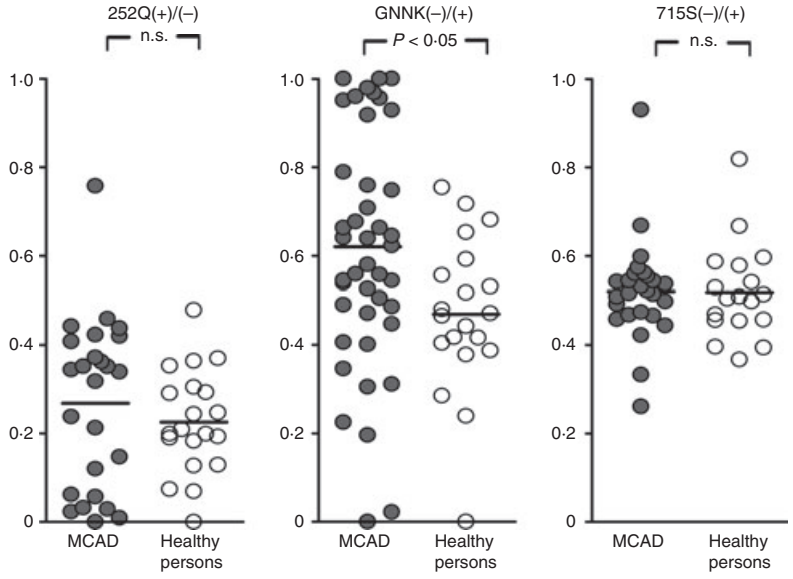

Figure 1. Distribution of the following ratios: (i) of Q252(+)/Q252(−) expression intensity (i.e. the amount of PCR amplification product with and without the additional glutamine residue [Q]); (ii) of the GNNK(−) isoform compared to the GNNK(+) isoform [i.e. the amount of PCR amplification product without and with the tetrapeptide sequence glycine-asparagine-asparagine-lysine (GNNK)]; and (iii) of S715(−)/S715(+) expression intensity (i.e. the amount of PCR amplification product with and without the serine residue [S]). Comparison of the distribution of the ratios between patients with systemic mast cell activation disease (MCAD, filled symbols) and healthy probands (healthy persons, open symbols). Ordinate: Ratio of the expression intensities. The black bars represent the respective mean. $P < 0{\cdot}05$, Fisher's exact test; n.s., not significant.

The D816V Kit mutation is present to a varying degree in patients with myeloid and lymphoproliferative mastocytosis-associated clonal haematological non-mast cell lineage diseases.[49,50] The occurrence of this mutation in these diseases points to a common bi-committed neoplastic progenitor clone. In several studies, the *KIT* mutation at codon 816 in mast cells was also detected on $CD34^+$ haematopoietic cells, eosinophils, monocytic neutrophil-lineage bone marrow precursor cells, and lymphocytes in around 30% of patients with SM.[28,51]

Genetic polymorphisms associated with Kit mutations

Kit^{D816V}-positive SM has been associated with a polymorphism in the *IL13* promoter gene (Table 2).[52] This polymorphism leads to a high transcription rate. Hence, the known proliferation-promoting influence of interleukin-13 (IL-13) on mast cells[53] should be enhanced. In accordance with this hypothesis, this polymorphism has been correlated with an elevated serum tryptase level, which is a marker of increased mast cell activation in SM.[4]

Daley *et al.*[54] reported that the polymorphism Q576R in the cytoplasmic domain of the α-subunit of the IL-4 receptor (Table 2) was found more frequently in SM patients whose disease was limited to skin, and who exhibited lower levels of surrogate disease markers. These data suggest that this polymorphism may mitigate disease expression, which may be a result of an increase in the efficacy of IL-4 at the mutated receptor. In accordance with this hypothesis, the addition of IL-4 to mast cell cultures has been shown to induce apoptosis.[55]

Finally, associations between SM and polymorphisms in *TET2* (a candidate tumour-suppressor gene), the *TNFα* gene, and the *VEGFA* promoter gene have been reported. No association with SM was found for *IL8* (Table 2).

However, none of the findings from the above mentioned candidate gene studies have been consistently replicated in independent studies, and so these findings cannot be regarded as proven.

Genome-wide gene expression profiling of human mast cells carrying Kit^{D816V}

D'Ambrosio *et al.*[56] obtained gene expression profiles from bone marrow mononuclear cells of eight patients with SM and the Kit^{D816V} mutation, and compared them with those of five healthy controls. Analysis of global median differential expression defined 130 genes with a known functional product that were differentially and significantly expressed in SM patients compared with controls. This suggests that SM is accompanied by an altered gene expression profile in the bone marrow. The most prominent of these differentially expressed genes was tryptase, which showed a 44·6-fold increase compared with controls. Genes involved in cell proliferation, neoplastic transformation, and apoptosis were also up-regulated. However, these findings require confirmation in experiments on purified mast cells from patients with SM and MCAS.

Table 2. Genes and genetic alterations related to systemic mast cell activation disease.

Gene	Function	Genetic alteration	Cells investigated	Summary of findings	Reference
KIT	Tyrosine kinase Kit	Multiple alterations	Mast cells and multiple other haematopoetic lineages	Clearly associated with pathological mast cell activation	Present paper
JAK2	Janus kinase 2	V617F	BMMC	Associated with pathological mast cell activation	79,80
PDGFRα	Tyrosine kinase platelet-derived growth factor receptor α	Fusion of FIP1L1 gene to PDGFRα gene in exon 12	PBMC; BMMC; multiple other haematopoetic lineages including mast cells	Associated with pathological mast cell activation	81–84
NRAS	Protein with intrinsic GTPase activity	G12D G13D	BMMC	Aggressive systemic mastocytosis	85
RASGRP4	Ras guanyl nucleotide-releasing protein	Failure to remove intron 5	Bone marrow mast cells	Systemic mastocytosis	35
CBL	E3-ligase	Multiple alterations	Retroviral expression of Cbl mutants in transplanted bone marrow in mice	Cytokine-independent mast cell activation	86
HRH4	Histamine H_4 receptor	Truncated splice variants	Cord blood-derived mast cells; eosinophils; basophils; dendritic cells	Inhibition of full-length histamine-H_4-receptor function	87
IL13	Interleukin 13	−1112C/T	PBMC	Association with Kit^{D816V}-positive systemic mastocytosis	52
IL4	Interleukin 4	Q576R	PBMC	Association with Kit^{D816V}-positive systemic mastocytosis	54
TET2	Candidate tumour suppressor gene	Multiple	BMMC; cord blood-derived mast cells and eosinophils	Association with Kit^{D816V}-positive systemic mastocytosis	88,89
TNF	Tumour necrosis factor α	238G/A	No information provided	Association with Kit^{D816V}-positive systemic mastocytosis	90
VEGFA	Vascular endothelial growth factor	−1154A/G	No information provided	Association with Kit^{D816V}-positive systemic mastocytosis	91

BMMC, bone marrow mononuclear cells; PBMC, peripheral blood mononuclear cells/leucocytes.

Cytogenetic findings associated with the Kit^{D816V} mutation

In a third of SM patients, genetic instability of the haematopoietic cells is reflected in chromosome abnormalities, as detected during cytogenetic analysis of bone marrow cells.[57–59] These abnormalities include deletions of chromosomes 5, 7, 11 and 20. No correlation between clinical features and cytogenetic findings has been reported.

Genetic alterations beyond Kit

Research has demonstrated induction of SM and MCAS secondary to mutations in the genes encoding the kinases JAK2 and PDGFRα; the Src-kinases; and proteins of cellular signal transduction (RASGRP4, *CBL*-encoded E3 ligase, histamine H_4 receptor; Table 2, references therein).

Heritability of systemic MCAD

Although numerous reports of familial occurrence and concordant twin pairs have strongly suggested a genetic basis for CM,[16,17,60–67] few data are available concerning the genetic contribution to systemic MCAD. To date, systemic MCAD has been assumed to be largely sporadic in nature[15], and only three familial cases of systemic MCAD (two SM and one MCAS) have been reported. In one SM family, the functionally activating point mutation Kit^{K509I} was identified in both affected patients (mother and daughter).[68] In a second SM family, a functionally activating mutation in Kit exon 8, which resulted in the deletion of codon 419, was detected in the three affected patients (mother, daughter, granddaughter) but not in the healthy sister.[69] One MCAS family has also been reported in which the father and his two children (daughter and son) presented with varying clinical features.[70] However, the underlying genetic alterations were not investigated.

Familial clustering of systemic MCAD

Demonstration of familial clustering would be an important step towards defining the genetic contribution to the risk of systemic MCAD. However, at the time of writing, no systematic investigation of the heritability of systemic

MCAD has been reported. Therefore, the present authors analysed data from 120 Caucasian index patients (Figure 2) with MCAS ($n = 102$) or SM ($n = 18$), who presented to our research group between May 2005 and November 2010, to determine familial aggregation. A sample of 258 probands was used as a control group representative of the German population. These individuals had been randomly recruited from the German inhabitants of the city of Bonn. Analysis of our data revealed that irrespective of systemic MCAD variant and gender, around 75% of our index patients had at least one first-degree relative with systemic MCAD (Figure 2). Within any given family, the disease variant, the severity of the mediator-related symptoms, and the age at clinical onset differed between individuals. Similar findings were reported in abstracts by Burks *et al.* 2005 and Bursztein *et al.* 2009.[70,71] The prevalence of systemic MCAD among the first-degree relatives in our sample was 33% (Figure 2), which differed significantly ($P < 0{\cdot}0001$; Fisher's exact test) from the prevalence in the control group (around 14%; Figure 2). The age distribution was similar in both groups. In both patients and controls, a female preponderance was observed (Figure 2). Our data suggest that systemic MCAD pedigrees include more systemic MCAD cases than would be expected by chance. This finding is consistent with the hypothesis that systemic MCAD has a heritable component, at least in a considerable number of patients.

At the time of writing, the molecular processes that result in a familial aggregation of MCAD remain speculative. The detection of differing systemic MCAD-associated somatic mutations within given families (refs 18,72; own unpublished data) is compatible with the hypothesis that disease arises secondary to a dysfunction of mutated, and as yet unidentified, operator or regulator genes. This dysfunction could result in mutations in *KIT* and other mast-cell-regulatory genes. Within this context, it is interesting to note that most of the insertions and deletions in *KIT* in patients with MCAS involve intron–exon junctions, suggesting that distortion of alternative splicing may contribute to the generation of these novel transcripts.[26]

Conclusions and perspective

Despite increasing understanding of its pathophysiology, the aetiology of MCAD remains largely unknown. The establishment of an aberrant clonal mast cell population is dependent upon mutations in kinases (particularly in the tyrosine kinase Kit) and in enzymes and receptors crucially involved in the regulation of mast cell activity. Other genetic alterations, some of which are as yet undetermined, appear to be related to systemic MCAD phenotype and prognosis.

Recent findings suggest that the various systemic MCAD variants and clinical phenotypes do not represent distinct disease entities, but rather varying presentations of a

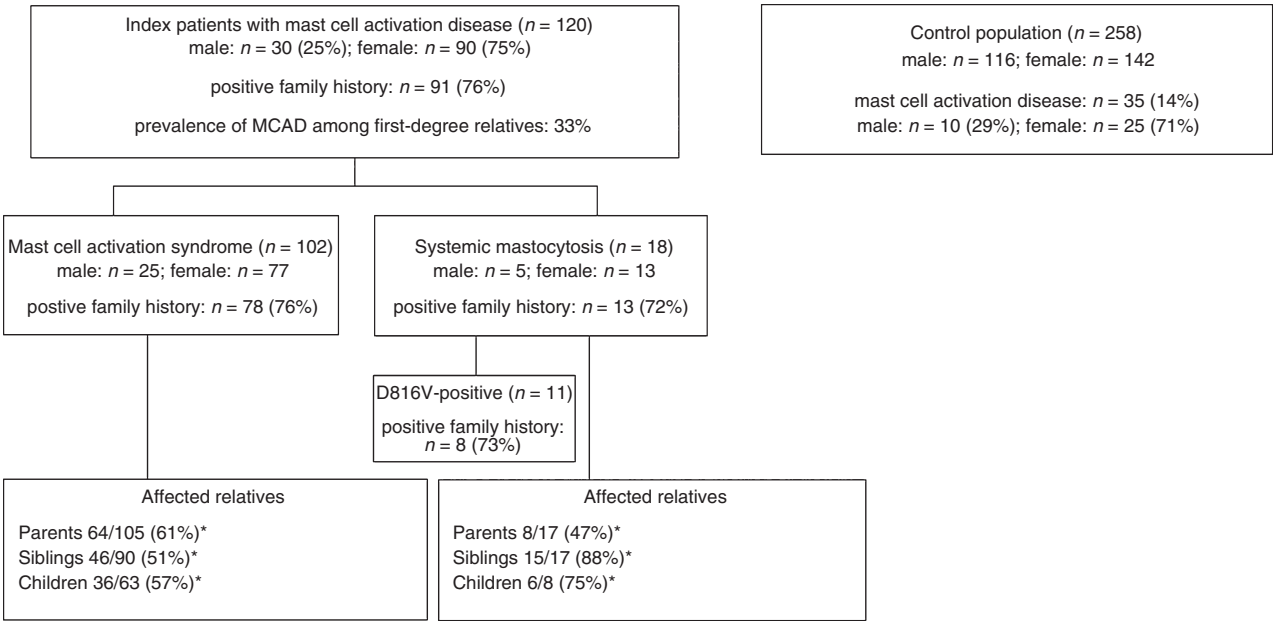

Figure 2. Characteristics of the presently investigated population in terms of the familial aggregation of systemic mast cell activation disease (MCAD). In the index patients, the diagnosis was assigned according to the criteria for MCAD.[1] Due to the anonymous nature of the survey of the control population, clinical diagnoses were assigned in the control group on the basis of a self-report checklist and the exclusion of relevant differential diagnoses (for the checklist, see ref. 92). Asterisks: number of affected persons/total number of persons in the respective group (percentage). A sample of 258 probands randomly recruited from the German inhabitants of the city of Bonn was used as a control group representative of the German population.

common generic root process of mast cell dysfunction.[2,7,25,26] Interestingly, in contrast to adult-onset systemic MCAD, more than 50% of paediatric cases of cutaneous mastocytosis appear to enter long-term remission spontaneously,[23,65] though whether such remissions are permanent or relapse in adulthood as systemic MCAD is unknown. The reason for this difference in prognosis – if both are clonal diseases with common roots – awaits clarification. Improved understanding of the biochemical processes leading to this resolution may open new avenues in the treatment of adult systemic MCAD.

To optimize research into the genetic basis of MCAD, a number of approaches may be suitable. First, next-generation sequencing could facilitate identification of the putative mutated disease-related operator and regulator genes in familial cases. Second, studies of animal models displaying disturbances secondary to primary mast cell dysfunction will implicate further genes of relevance to systemic MCAD. In dogs, mast cell tumours are the second most common form of neoplastic disease (7–21% of all cutaneous neoplasms). In up to 40% of all canine mast cell tumours, *KIT* mutations are present. Well-documented breed predispositions indicate an underlying heritable component (for review, see ref. 73). Third, genome-wide association studies could allow the unbiased and systematic identification of systemic MCAD risk genes. Since their introduction, genome-wide association studies have identified risk genes for several multifactorial diseases (http://www.genome.gov/gwastudies/). However, these studies showed that most risk genes have only moderate effect sizes, and that analysis of large samples is necessary. Given the high prevalence of the MCAS variant, the recruitment of such samples should be possible at least for MCAS.

Taken together, improved knowledge of the genetic aetiology of MCAD may facilitate the development of new therapeutic strategies, which possibly may also imply curative in addition to symptomatic treatment options.

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Disclosures

The authors declare that they have no competing interests.

Authors' contribution

All authors contributed equally to conception, design and drafting of the manuscript. All authors read and approved the final manuscript.

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